iGEM TU/e 2014
Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2014.igem.org/Team:TU_Eindhoven

Date
4 August 2014

Protocol Droplet Separation

This is a droplet separation protocol for the extraction of polyacrylamide droplets. Two methods of separation – centrifugation and filtration – are used as can be seen below. In the TU Eindhoven iGEM team, centrifugation appeared be more effective than filtration. The protocol has been composed with the use of scientific resources and is improved by trial and error during the experiments.

Technische Universiteit
Eindhoven
University of Technology

Table of contents

Title
Droplet Separation

1 Centrifugation I

- Take the eppendorf tube where the droplets are stored.
- Add with 20% v/v of PFO (Perfluoroctane) in the HFE-7500.
- Gently mix the PFO with the oil phase by tapping the eppendorf cup.
- Leave the cup on the work bench for 1-2 minutes. Once the droplets are broken, the two phases become clear and the beads are separated from the oil phase.
- Spin the tube at 60 × g for 30 seconds to fully separate the two phases.
- Add distilled water on a 1:1 ratio.
- Since the droplets are located on the separation layer of the oil and water phase, pipet the oil phase away until only the water (with the droplets) is present in the eppendorf tube.
- Put 5 µL of the solution (pipet on the bottom of the eppendorf tube) on a glass slab to analyze further under the microscope. Cover the solution sample with a coverslip.

2 Separation

- Remove the mineral oil from the top of the collected particles.
- Centrifuge at 3000 × g for 30 seconds.
- Remove bottom oil phase.
- Add 500 µL of neat HFE-7500, vortex and centrifuge.
- Remove bottom oil phase.
- Add 500 µL of HFE-7500 with 20% v/v perfluorooctane, vortex and centrifuge.
- Remove bottom oil phase.
- Add 1 mL 1 w/t% span-80 in hexanes, vortex and centrifuge.
- Remove upper hexane phase.
- Add 1 mL 0.1 w/t% triton X-100 in water.
- Pipette up and down, vortex or sonicate to disperse particles, centrifuge.
- Remove upper water phase including any oil/water emulsion floating on top of the liquid.
- Add 1 mL 0.1 w/t% triton X-100 in water.
- Pipette up and down, vortex or sonicate to disperse particles, centrifuge.
- Disperse in plain water.

3 References

Mazutis, L., Gilbert, J., Ung. W.L., Weitz, D.A., Griffiths, A.D. & Heyman J.A. (2013). Single-cell analysis and sorting using droplet-based microfluidics. *Nature, 8(5),* pp. 870-91.

Diamante, L., Gatti-Lafranconi, P., Schaerli Y. & Hollfelder, F. (2013). In vitro affinity screening of protein and peptide binders by megavalent bead surface display. *Protein Engineering, Design & Selection, 26(10),* pp. 713-724.